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The Effects of REM Sleep Deprivation on Striatal Dopamine Receptor Sites[1]

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ZWICKER, A P AND H M. CALIL. *The effects of REM sleep deprivation on striatal dopamine receptor sites* PHARMACOL BIOCHEM BEHAV 24(4) 809–812, 1986 —^{3}H-Spiroperidol binding to dopamine receptor sites of rat striatal tissue was studied following 24, 48, 72 and 96 hr of rapid eye movement sleep deprivation (REM dep.) The density of dopamine receptor binding sites (B_{max}) was decreased after 48, 72, and 96 hr of REM dep. The apparent dissociation constant (K_D) decreased after 96 hr, indicating an increase in apparent affinities The control experimental animals also presented a time-dependent decrease of B_{max} and K_D as compared to unhandled controls These results suggest that dopaminergic mechanisms may indeed be involved in the effects of REM sleep deprivation and/or stress

REM sleep deprivation Rat striatum ^{3}H-Spiroperidol binding Dopamine receptor

SEVERAL chronic antidepressant treatment modalities (tricyclic antidepressants, monoaminoxidase inhibitors, electroconvulsotherapy) decrease the density of β-adrenergic receptor sites in rat cerebral cortex (for review see [4]).

Rapid eye movement sleep deprivation (REM dep.) , frequently followed by improvement of depression in man [23], also decreased the number of β-receptor binding sites of rat cerebral cortex [12]. However, this effect of REM dep. was not replicated in other studies [1,15]

The effects of REM dep. on the dopaminergic system have been extensively studied behaviorally The results show an increased response to directly and indirectly acting dopamine (DA) agonists, leading to the suggestions that such hyperresponsiveness might reflect either postsynaptic dopamine receptor supersensitivity [3, 13, 21] or presynaptic receptor subsensitivity [17]. However, the neurochemical effects of sleep deprivation have not been extensively studied One recent study did not find changes in either the number (B_{max}) or affinity (K_D) of dopaminergic postsynaptic D_2 sites in striatum and frontal cortex following 96 hr sleep dep Similarly, presynaptic receptor sensitivity, as measured by apomorphine-induced inhibition of tyrosine hydroxylase, was unchanged in striatum [7].

The present study systematically investigated the possible alterations of striatal dopaminergic receptor sites in the rat following several periods of REM dep.

METHOD

Male Wistar rats (200–300 g), housed three to a cage in an air-conditioned room with a 12 hr light-dark cycle (lights on at 7 a.m.), and having free access to food and water were used REM sleep deprivation (REM dep.) was achieved with the "flower pot" technique [11], which consists of placing the rats on small platforms (7 cm in diameter) surrounded by water in a metal container—this condition allows the occurrence of slow wave sleep but substantially reduces the amount of REM sleep An experimental control group was run simultaneously, in order to control for the stress inherent to the REM dep. procedure (isolation and immobilization on the platform) The rats were then placed on larger platforms (14 cm in diameter), which allows REM sleep without the rats falling in the water REM dep. and control groups were submitted to this procedure during 24, 48, 72 and 96 hr. Several previous EEG recording studies were run to ensure that rats placed on small platforms were REM sleep deprived whereas those on larger platforms were not (for review see [22]). Finally normal control rats were maintained in their home-cages without any manipulation except for routine care every morning.

Rats were sacrificed by guillotine decapitation at 0 (normal control group), 24, 48, 72 and 96 hr (experimental groups) after beginning of the experimental procedure Their brains were quickly removed on ice, and the striata were dissected, weighed, and homogenized. The striata were assayed for DA receptor binding according to the method of Burt *et al* [2] with the following small modification. The striatal tissue was homogenized in 100 vol. (w/v) of ice-cold Tris buffer (0.05 M, pH 7 7) and centrifuged (Beckman Mod. L5-50) at 20000 g for 10 min. After two 100-vol. washes in ice-cold Tris buffer, the membranes were resuspended to a final concentration of 100 mg striatum/10 ml buffer. Then, aliquots of 900 μl (9.0 mg striatum) homogenate were incubated at 37°C for 10 min with 100 μl of ^{3}H-spiroperidol (New

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England Nuclear, 31.3 Ci/mmol) at 6 concentrations, ranging from 0 0625 to 1.0 nM or with ^{3}H-spiroperidol and 20 μl of 10 μM dextrobutaclamol (Ayerst Research Laboratories) prepared with 0 1% ascorbic acid. The concentrations were chosen from previous studies employing a larger concentration range (up to 5 nM) to separate low and high affinity binding sites The samples were run in duplicates After incubation, samples were filtered (Whatman GF/B 2 4 cm glass fiber filters) under vacuum with three 6 ml washes of cold Tris (pH 7 0) buffer. Dried filters were transferred to vials containing 5 ml Aquasol for later counting in a Beckman Scintillation Counter (Mod LS-3150T) Specific binding was defined as the difference between total binding and the binding occurring in the presence of 10 μM dextrobutaclamol binding

The maximal number of receptor sites (B_{max}) and the dissociation constant (K_D) values were obtained by Scatchard analysis [16] using a computer program employing linear regression analysis.

Data (expressed as mean±SEM) of control and experimental groups were evaluated using an analysis of variance (ANOVA) followed by Duncan's new multiple range test The comparison between experimental groups for the different time periods was done by Student's t-test for independent samples

RESULTS

The specific binding of ^{3}H-spiroperidol, in concentrations ranging from 0.0625 to 1.0 nM, to striatal dopaminergic receptor sites represented 66 6 to 76 8% of the total binding. Results obtained from the initial studies through the Scatchard analysis showed that the B_{max} was 39.26 pmol/g wet weight of tissue and the K_D was 1 52 nM (Fig. 1). Data were also analysed with the Hill plot, which revealed a straight line with a slope equal to unity

The B_{max} and K_D mean values for all groups are shown in Table 1.

The B_{max} values were decreased ($p<0$ 01) following 48, 72 and 96 hr REM dep. (small platforms), and 72 and 96 hr of experimental control (larger platforms) ($p<0.05$) in comparison with normal controls There were also reductions of B_{max} values after 72 and 96 hr REM sleep dep ($p<0.05$) in comparison with the 24 hr experimental group In addition, a concomitant decrease of K_D ($p<0$ 01) as compared to normal controls was found following 96 hr of the experimental procedure in both groups, thus indicating an increase of the apparent affinities between ligand and receptor sites Furthermore, a slight increase of K_D occurred 24 hr after beginning of the experimental procedure, again compared to the normal controls Although this increase was not significantly different from the normal controls, it was higher than the K_D observed 48, 72 ($p<0$ 05) and 96 hr ($p<0$ 01) for both experimental groups

DISCUSSION

Forty-eight or 96 hr of REM sleep deprivation was sufficient to induce significant decrease of B_{max} and K_D. These data are different from the findings of two previous studies [7,25] The Wirz-Justice study analysed the effects of sleep deprivation on the circadian rhythms of rat brain neurotransmitter receptors, and reported an increase in the number of dopaminergic receptor sites during sleep deprivation in the dark phase (last 13 hr of a 24 hr sleep dep period).

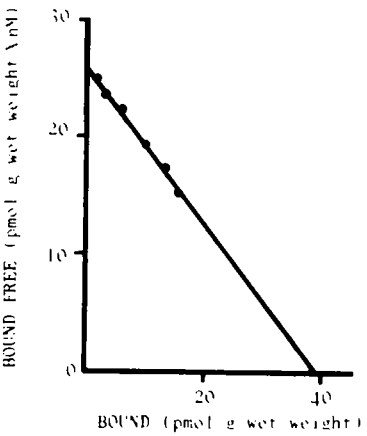

FIG 1 Scatchard analysis of ^{3}H-spiroperidol (0 0625–1 0 nM) specific binding to dopamine receptor sites in rat striata (as described in the Method section) K_D=1 52 nM and B_{max}=39 26 pmol/g wet weight Each point is the mean of duplicate determinations

The present study found a slight decrease (11%) of B_{max} following 24 hr REM dep This discrepancy could possibly be explained by differences in experimental procedures utilized to achieve sleep deprivation in both studies In contrast to the present and the Wirz-Justice studies, Farber [7] did not find B_{max} and K_D changes in the striatum and frontal cortex of rats following 96 hr REM dep This latter study employed a REM sleep deprivation procedure similar to the one in the present study, but a different binding assay method which included a higher concentration range of ^{3}H-spiroperidol (up to 2 0 nM) It has been described that higher concentrations of ^{3}H-spiroperidol also bind to low affinity sites [20]

It is interesting to note that studies of β-receptor sites in rat cerebral cortex showed similar inconsistent results as those found for dopaminergic receptor sites Thus, it was reported that 72 hr REM dep. induced besides a decrease of B_{max}, a significant reduction of K_D [12] These results also have not been replicated in rats submitted to either 72 hr or seven day REM sleep deprivation [1,15] The decrease of β-adrenergic receptor site number following chronic administration of other antidepressant treatment modalities (tricyclic, MAO inhibitors, electroconvulsive therapy) is not accompanied by K_D alterations (for review see [4]) It is thus possible that, at least in this aspect, REM sleep deprivation of laboratory animals may be qualitatively different from other antidepressant treatments Clinically, it has been reported that selective REM sleep deprivation requires chronic rather than acute (24 hr) application before inducing a therapeutic response [23,24]

The decrease of B_{max} and K_D in the experimental control group, also occurred in a time-dependent way, though it was quantitatively smaller than the changes seen in the REM sleep deprived group Similar results have been described for β-adrenergic receptor sites [12] This change (decrease) could be associated with other variables inherent to the experimental procedure, such as isolation of the animal, motor restriction, and humidity Consequently, taking these variables into account, a possible influence of stress at the level of dopaminergic and/or noradrenergic receptor sites can not be ruled out Moreover, behavioral studies have shown that rats submitted to REM sleep deprivation or to foot shock for two

TABLE 1

EFFECTS OF ISOLATION ON LARGE PLATFORMS (EXPERIMENTAL CONTROLS) AND OF REM SLEEP DEPRIVATION ON ^{3}H-SPIROPERIDOL BINDING TO RAT STRIATAL DOPAMINERGIC RECEPTOR SITES

	N	B_{max}(pmol/g wet weight)	% of B_{max} Decrease Compared to Normal Controls	K_D (nM)
Normal controls	6	38 00 ± 2 76	—	1 50 ± 0 07
Experimental controls				
24 hr	5	36 56 ± 5 72	4	1 69 ± 0 10
48 hr	7	32 32 ± 2 72	15	1 42 ± 0 08§
72 hr	5	25 85 ± 2 71†	32	1 38 ± 0 10§
96 hr	8	27 35 ± 2 04†	28	1 15 ± 0 06*‡
REM sleep deprived				
24 hr	5	33 95 ± 3 81	11	1 66 ± 0 12
48 hr	7	27 08 ± 1 84*	29	1 29 ± 0 12§
72 hr	5	23 48 ± 3 73*§	38	1 15 ± 0 17§
96 hr	8	25 07 ± 1 32*§	34	1 07 ± 0 09*‡

Duncan's new multiple range test *($p \leq 0$ 01), †($p \leq 0$ 05) in comparison to normal controls, ‡($p \leq 0$ 01), §($p \leq 0$ 05)· compared to the 24 hr experimental controls or REM sleep deprived

Data are expressed as mean ± standard error of the mean (mean ± SEM) of the maximal number of receptor sites (B_{max}), and of the dissociation constant (K_D) N=number of experiments for each group

or three days, presented more apomorphine induced aggressiveness than normal control rats [18]. Also the aggressiveness, as measured by shock-induced fighting, was twice the control level in REM sleep deprived rats for seven days. This latter study also found a 30% decrease in the number of cortical β-adrenergic receptor sites [6]. In spite of these findings, it has already been well documented that these alterations seem to be more related to REM sleep deprivation since they are more pronounced in REM sleep deprived animals than in the experimental controls (see [22] for review). The decrease in apparent affinities between ligand and receptor sites, as indicated by a discrete K_D increase seen at 24 hr after beginning of the experimental procedure, coincided with the initial drop in receptor site number.

The observed decrease of dopaminergic receptor sites (D2)—a subsensitivity—is apparently opposite to the increase of behavioral responsiveness—supersensitivity—induced by dopaminergic stimulation in animals previously deprived of REM sleep. It may be speculated that the increase in apparent affinity (decreased K_D) could be a compensatory change as a consequence of the decrease of these sites. Could this K_D change explain the behavioral supersensitivity? It is possible to suppose that an affinity change could override the decrease in receptor site number.

Another possible explanation for the discrepancies in the effects of REM sleep deprivation at neurochemical and behavioral levels would be the existence of different types of dopaminergic receptors [5,9]. The present study utilized only one ligand, considered to be specific for D2 type receptor sites [10]. However, most behavioral studies (e.g., stereotypy, aggressiveness) utilizes an agonist which binds to both postsynaptic and autoreceptor or D1 and D2 dopaminergic binding sites [8, 14, 19]. Future studies should be done with other ligands, agonists and antagonists to further elucidate the neurochemical effects of REM sleep deprivation on DA systems.

REFERENCES

1 Abel, M S , F Villegas, J Abreu, F Gimino, S Steiner, B Beer and L R Meyerson The effect of rapid eye movement sleep deprivation on cortical β-adrenergic receptors *Brain Res Bull* **11**: 729–734, 1983

2 Burt, D R , I Creese and S H Snyder Properties of ^{3}H-haloperidol and ^{3}H-dopamine binding associated with dopamine receptors in calf brain membranes *Mol Pharmacol* **12**: 800–812, 1976

3 Carlini, E A REM sleep deprivation and dopamine in the CNS *Rev Pure Appl Pharmacol Sci* **4**: 1–25, 1983

4 Charney, D S , D B Menkes and G R Heninger Receptor sensitivity and the mechanism of action of antidepressant treatment implications for the etiology and therapy of depression *Arch Gen Psychiatry* **38**: 1160–1180, 1981

5 Costall, B and R J Naylor The hypotheses of different dopamine receptor mechanisms *Life Sci* **28**: 215–229, 1981

6 Eichelman, B and L R Hegstrand Stress-induced alterations in aggression and brain chemistry Abstract of 13th CINP Congress, Vol I, Israel, 1982, p 190

7 Farber, J , J D Miller, K A Crawford and B A McMillen Dopamine metabolism and receptor sensitivity in rat brain after REM sleep deprivation *Pharmacol Biochem Behav* **18**: 509–513, 1983

8 Gershanik, O , R E Heikkila and R C Duvoisin Behavioral correlations of dopamine receptor activation *Neurology* **33**: 1489–1492, 1983

9 Grigoriadis, D and P Seeman The dopamine/neuroleptic receptor *Can J Neurol Sci* **11**: 108–113, 1984

10 Hamblin, M W, S E Leff and I Creese Interactions of agonists with D-2 dopamine receptors evidence for a single receptor population existing in multiple agonist affinity-states in rat striatal membranes *Biochem Pharmacol* **33**: 877–887, 1984
11 Mendelson, W B, R D Guthrie, G Frederick and R J Wyatt The flower pot technique of rapid eye movement (REM) sleep deprivation *Pharmacol Biochem Behav* **2**: 553–556, 1974
12 Mogilnicka, E, S Arbilla, H Depoortere and S Z Langer Rapid-eye-movement sleep deprivation decreases the density of ^{3}H-dihydroalprenolol and ^{3}H-imipramine binding sites in the rat cerebral cortex *Eur J Pharmacol* **65**: 289–292, 1980
13 Mogilnicka, E REM sleep deprivation changes behavioral response to catecholaminergic and serotonergic receptor activation in rats *Pharmacol Biochem Behav* **15**: 149–151, 1981
14 Murugaiah, K, S Fleminger, M D Hall, A Theodorou, P Jenner and C D Marsden Alterations in different populations of striatal dopamine receptors produced by 18 months continuous administration of cis- or trans-flupenthixol to rats *Neuropharmacology* **23**: 599–609, 1984
15 Radulovacki, M and N Micovic Effects of REM sleep deprivation and desipramine on β-adrenergic binding sites in rat brain *Brain Res* **235**: 393–396, 1982
16 Scatchard, G The attractions of proteins for small molecules and ions *Ann NY Acad Sci* **51**: 660–672, 1949
17 Serra, G, M R Melis, A Argiolas, F Fadda and G L Gessa REM sleep deprivation induces subsensitivity of dopamine receptors mediating sedation in rats *Eur J Pharmacol* **72**: 131–135, 1981
18 Silva, C C, F Campos and E A Carlini Aggressive behavior induced by apomorphine in rats submitted to four stressful situations *Res Commun Psychol Psychiatr Behav* **5**: 353–367 1980
19 Sokoloff, P, M P Martres and J C Schwartz ^{3}H-Apomorphine labels both dopamine post-synaptic receptors and autoreceptors *Nature* **288**: 283–286, 1980
20 Sundermann, R H and G F Wooten Biochemical properties of spiperone binding to rat brain membranes *Pharmacology* **21**: 295–305, 1980
21 Tufik, S, C J Lindsey and E A Carlini Does REM sleep deprivation induce a supersensitivity of dopaminergic receptors in the rat brain? *Pharmacology* **16**: 98–105, 1978
22 Vogel, G W A review of REM sleep deprivation *Arch Gen Psychiatry* **32**, 749–761, 1975
23 Vogel, G W, A Thurmond, P Gibbons, K Sloan, M Boyd and M Walker REM sleep reduction effects on depression syndromes *Arch Gen Psychiatry* **32**, 765–777, 1975
24 Vogel, G W, F Vogel, R S McAbee and A J Thurmond Improvement of depression by REM sleep deprivation new findings and a theory *Arch Gen Psychiatry* **37**: 247–253, 1980
25 Wirz-Justice, A, I Tobler, M S Kafka, D Naber, P J Marangos, A A Borbély and T A Wehr Sleep deprivation effects on circadian rhythms of rat brain neurotransmitter receptors *Psychiatry Res* **5**: 67–76, 1981